

Reply to Smith et al.: No evidence to challenge the current paradigm on starch and cellulose biosynthesis involving sucrose synthase activity

The generally accepted view that sucrose synthase (SUS) activity is involved in the production of cellulose and starch biosynthesis (1 and references contained therein) has been challenged (2). Using a method for assaying SUS activity in the synthetic direction (conversion of UDP-glucose into sucrose), Barratt et al. (2) reported an almost total absence of SUS activity in *sus1/sus2/sus3/sus4* *Arabidopsis* mutant stems and the presence of WT levels of starch and cellulose content in this organ. Bieniawska et al. (3) reported that SUS activities in WT *Arabidopsis* leaves were too low to account for the starch accumulation rate occurring during illumination. Barratt et al. (2) thus concluded that SUS is not required for starch and cellulose biosynthesis in *Arabidopsis*. However, in our study (1), we used a method for assaying SUS activity in the sucrose cleavage direction (UDP-glucose and ADP-glucose synthesis) and found that SUS activity in WT *Arabidopsis* leaves was approximately 10-fold higher than that reported by Bieniawska et al. (3), greatly exceeding the minimum needed to support the normal rate of starch accumulation during illumination. Furthermore, we found that SUS activities in the insoluble and soluble fractions of *sus1/sus2/sus3/sus4* stems were approximately 10- and 100-fold higher, respectively, than those reported by Barratt et al. (2). Most importantly, we also found that SUS activity in the leaves and stems of the *sus1/sus2/sus3/sus4* mutants was approximately 85% of those of WT plants, thus concluding that (i) most of SUS activity in *Arabidopsis* leaves and stems is not attributable to SUS1–4 and (ii) SUS activity in *sus1/sus2/sus3/sus4* mutants is sufficient to support normal cellulose and starch biosynthesis. As discussed in our paper (1), we ascribed such marked differences between our results and those of Barratt et al. (2) and Bieniawska et al. (3) to the inadequate method and conditions for assaying SUS activity used by these investigators rather than to differences in the assay temperature. In their letter, Smith et al. (4) surmise that the method for assaying SUS

activity should not distract attention when formulating conclusions about the role of SUS in starch and cellulose metabolism, and asserted that we ignored their immunolocalization studies of SUS1–6. However, we consider that the use of an inadequate method for assaying SUS activity is not a minor issue, because conclusions by Barratt et al. (2) and Bieniawska et al. (3) were strongly based on the use of such methods. Studies of SUS1–6 location conducted by Barratt et al. (2) and Bieniawska et al. (3) were carried out assuming the yet to be demonstrated conceptual idea that SUS1–6 are the sole proteins with SUS activity in *Arabidopsis*. Therefore, as indicated in our paper (1), further endeavors based on the production of plants totally lacking SUS activity and on the use of adequate SUS activity assay method(s) will be necessary to investigate the involvement of SUS activity in starch and cellulose metabolism. In conclusion, in our opinion, no pressing biological evidence has been presented by Barratt et al. (2) to challenge the current paradigm on cellulose and starch metabolism involving SUS activity. In this context, we must emphasize that Angeles-Núñez and Tiessen (5) have shown that SUS2 and SUS3 are required for channeling carbon toward ADP-glucose and starch in *Arabidopsis* seeds.

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4. Smith AM, Kruger NJ, Lunn JE (2012) Source of sugar nucleotides for starch and cellulose synthesis. *Proc Natl Acad Sci USA* 109:E776.
5. Angeles-Núñez JG, Tiessen A (2010) *Arabidopsis* sucrose synthase 2 and 3 modulate metabolic homeostasis and direct carbon towards starch synthesis in developing seeds. *Planta* 232:701–718.

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The authors declare no conflict of interest.

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